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Selectivity and Potency of Cyclin-dependent Kinase Inhibitors

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ABSTRACT

Members of the cyclin-dependent kinase (CDK) family play key roles in various cellular processes. There are 11 members of the CDK family known till now. CDKs are activated by forming noncovalent complexes with cyclins such as A-, B-, C-, D- (D1, D2, and D3), and E-type cyclins. Each isozyme of this family is responsible for particular aspects (cell signaling, transcription, etc) of the cell cycle, and some of the CDK isozymes are specific to certain kinds of tissues. Aberrant expression and overexpression of these kinases are evidenced in many disease conditions. Inhibition of isozymes of CDKs specifically can yield beneficiary treatment modalities with minimum side effects. More than 80 3-dimensional structures of CDK2, CDK5, and CDK6 complexed with inhibitors have been published. This review provides an understanding of the structural aspects of CDK isozymes and binding modes of various known CDK inhibitors so that these kinases can be better targeted for drug discovery and design. The amino acid residues that constitute the cyclin binding region, the substrate binding region, and the area around the adenosine triphosphate (ATP) binding site have been compared for CDK isozymes. Those amino acids at the ATP binding site that could be used to improve the potency and subtype specificity have been described.

KEYWORDS: cyclin-dependent kinases, cell cycle, CDK inhibitors, structure-based design/discovery, ATP binding site, cyclin binding peptides

INTRODUCTION

Protein kinases play important regulatory roles in diverse cellular processes, such as metabolism, transcription, cell cycle progression, apoptosis, and neuronal development.[1-3] Mutations that deregulate these kinases' function or expression, or both, result in a myriad of diseases, such as cancer,[4] neurodegenerative disorders[5] (eg, Alzheimer's disease, stroke), cardiovascular disorders, and viral infections. Protein kinases, now the focus of intense research efforts, have the potential to lead to new therapies for many diseases that challenge the medical community.[6,7] Five hundred eighteen protein kinases have been identified in the human genome.[8,9] These have been classified into 8 families based on the sequence comparison of their catalytic domains and their known biological functions. The cyclin-dependent kinases (CDKs), which are serine/threonine kinases, are grouped in the CMGC kinase family along with mitogen-activated kinases, glycogen-synthase kinases, and CDK-like kinases.

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Cyclin-dependent Kinases

There are 11 members of the CDK family known till now. Among these, CDK1, 2, 3, 4, and 6 are known to play important roles in the cell cycle.[10,11] CDK5 activity is restricted to the nervous system.[12] CDK7 plays an indirect role in the cell cycle by activating the CDKs involved in the cell cycle. CDKs 7, 8, and 9 are regulators of transcription.[13-15] Not much is known about the function of CDK10, but it has been shown to interact with transcription factor Ets2.[16] As suggested by their name, the CDKs associate with cyclins for their activation, except for CDK5, which associates with p35 and p39. Fifteen cyclins have been identified so far. Cyclins A and E activate with CDK2; cyclins D1, D2, and D3 associate with CDK4 and CDK6; cyclins A and B interact with CDK1; and cyclin H, with CDK7. The association of the CDKs with their requisite cyclin partner results in the CDKs adopting a substrate-specific catalytic subunit. The timing of expression of specific cyclins controls the activation of their interacting CDKs. These 2 aspects provide the kinase specificity, targeted function, and control of cell division, including transcription, DNA repair, and postmitotic changes. The CDKs are additionally controlled by phosphorylation, rigidly binding inhibitors, ubiquitin-mediated proteolysis of cyclins, and CDK inhibitors.

Cyclin-dependent Kinases, Cell Cycle, and Cancer

The division of eukaryotic cells progresses through 4 phases, gap 1 (G_1), synthetic (S), gap 2 (G_2), and mitosis (M), governed

by a series of proteins (ie, cyclins) by binding to and activating specific CDKs. G_0 is the resting state of the cells. Different cyclin/CDK pairs are active during each phase of the cell cycle. It has been shown recently that CDK3-cyclin C complex helps the cells to efficiently exit the G_0 state and enter the G_1 phase.[17] This action is accomplished by stimulation of retinoblastoma protein (pRb) phosphorylation at S807/811 by the CDK3-cyclin C complex during the G_0/G_1 transition. Mitogenic signals induce the transcription factors of the dormant cell (G_0 phase), and the cell enters the G_1 phase. CDK4/6 on binding to cyclin D begins the phosphorylation of pRb complexed to E2F/DP (transcription factors). Following pRb phosphorylation, cyclin E activates CDK2 to effect further phosphorylation of pRb, thereby enabling the cells to cross the G_1 restriction point. The pRb-E2F/DP complex disassociates, providing a positive signal for DNA synthesis in the S phase. Cyclin E is replaced by cyclin A, which binds to CDK2 and leads to phosphorylation of DP-1 subunits (inhibitor of DNA binding), CDC6 (initiator of DNA replication). On completion of the S phase, cyclin B-CDK1 complex (mitosis-promoting factor) is activated. Progression from G_2 to M phase requires sustained activity of CDK1-cyclin B complex within the nucleus. Subsequent entry into the anaphase relies critically on the sudden destruction of the CDK1-cyclin B activity, which guarantees the global inhibition of protein biosynthesis, DNA replication, and DNA transcription.[18,19] Cyclin H complexes with CDK7 to form the CDK-activating kinase (CAK).[20] When cyclins form 1:1 complexes with their cognate CDK catalytic subunits to form the CDK holoenzyme, CAK phosphorylates these complexes at specific residues, resulting in their activation. The importance of CDK-cyclin complexes for the progression of the cell cycle is generally accepted.

CDKs are activated by forming noncovalent complexes with cyclins such as A-, B-, C-, D- (D1, D2, and D3), and E-type cyclins. These complexes are responsible for the phosphorylation and inactivation of the retinoblastoma family of proteins, which is the key controller of the cell cycle.[21,22] Retinoblastoma protein contains multiple phosphorylation sites, and different CDKs show distinct site preference for phosphorylation of pRb.[23,24] Other substrates of CDKs include p107 and p130.[11] The CDKs also function as "docking sites" for a series of proteins that must be tightly regulated during the cell cycle.[25] In addition, CDK2 phosphorylates substrates that are involved in DNA replication.[26,27] CDKs are activated and regulated by cyclins, p25, CAK, KAP/CDI1, CDC25, etc. These regulations are achieved by binding to CDKs (eg, noncovalent binding of cyclins to CDKs), phosphorylating selective residues of CDKs (eg, phosphorylation of T160 on CDK2 by KAP/CDI1),[11,20,28] and removing inhibitory phosphorylation (in the case of CDK4, CDK6, CDK2, and CDK1 by CDC25).[11,28] Further control of CDKs is exerted by inhibitors that can be divided into 2 distinct families: the CIP/KIP and the INK4 families.[27,29,30] The first class of inhibitors, namely, the CIP/KIP family, comprising p21, p27, and p57, inhibit CDK2-cyclin E/A complexes by trimerization, leading to arrest of the cell cycle in the G_1 phase.[31] Proteins p21 and p27 act as both positive and negative regulators for the CDK4/6-cyclin D complexes. They are required for the metabolic stability and translocation to the nucleus of these complexes and for blocking the CDK4/6-cyclin D complexes when p21 and p27 are overexpressed.[27,32] The second class of inhibitors, INK4 inhibitors, consisting of p16, p15, p18, and p19 proteins, bind to CDK4/6, forming a trimeric complex and thereby preventing binding to the activator cyclin D. Thus, the inhibitors act in myriad ways to arrest the cell cycle at the G_1 phase.

Inappropriate cell cycle progression is the basic feature of human tumors. Mutations and overexpression of cyclins, and CDKs loss of expression of Rb and CKI are the hallmark of neoplasias, including B-cell lymphoma, T-cell lymphoma, esophageal cancer, breast cancer, bladder cancer, small cell lung cancer, colon cancer, glioblastoma, neuroblastoma, familial adenomatous polyposis, and familial melanoma. Cyclin D1 and cyclin E are often overexpressed in human breast and colon cancer as well as several other types of human cancer.[33-35] Amplification and overexpression of CDK4 are also seen in human cancers.[33,36] Phosphatases that activate cyclin-CDK complexes are frequently overexpressed in human breast cancers, non-small-cell cancers, and head and neck cancers.[37,38] Arrest of the cell cycle at the G_1/S phase is a necessary part of cancer chemoprevention or treatment. Inhibition of catalytic activity of the CDKs with the help of small molecules that compete with adenosine triphosphate (ATP) has proved to be the most successful strategy to date.

Cyclin-dependent Kinases, Apoptosis, and Neuronal Cell Death

While the essential role of CDKs in the cell cycle has been defined unequivocally, their effect on apoptosis has not been completely discerned. Different experimental conditions have produced different roles for CDKs, inducing apoptosis or protecting cells from apoptosis. It has been shown that PC12[39] cells in proliferation are not protected from apoptosis by CDK inhibitor but, in fact, promote apoptosis. In contrast, when cells were cultured in differentiating conditions, flavopiridol and olomoucine protected cells from apoptosis, implying that cellular context is the governing factor determining the role of CDKs in apoptosis. The CDK1-cyclin B complex is essential for entry into mitosis. Overexpression of CDK1-cyclin B complex is a component of maturation promoting factor, which is a major factor for chromosome

formation. Premature chromosome condensation leads to mitotic catastrophe, and overexpression of CDK1-cyclin B complexes has been reported in mitotic catastrophe. It has been shown that CDK1 phosphorylates BAD at the S128 position in apoptotic cells (CDK1 expression can be induced in cells that are outside the cell cycle), indicating that specific CDK1 activity is required for apoptosis. Forced overexpression of only CDK4 or cyclin D1 induces apoptosis in cultured cells with normal pRb. The role of CDKs in apoptosis needs to be more completely realized in order for CDK inhibitors to be used clinically, as the ultimate goal in fighting cancer would be destruction of the cancerous cells.

An exception among the CDKs is CDK5, which does not seem to be involved in cell cycle regulation even though it binds and phosphorylates pRb. Another unique aspect of CDK5 is that it is activated by p35 and its homolog p39 and not by any of the cyclins. Since p35 and p39 are expressed in only neurons, CDK5 activity is seen in only neurons despite its expression in most tissues. Researchers have identified many substrates of CDK5 that play pivotal roles in central nervous system (CNS) development, establishing the critical role of CDK5 in the normal development and function of the brain.[40] CDK5 plays a central role in neuronal migration during the development of the CNS.[41] Mice lacking CDK5 or double-deficient for its 2 brain activators, p35 and p39, exhibit severe defects in the layering of the cerebral cortex.[40] CDK5 regulates the actin and microtubules of the cytoskeleton and modulates cell adhesion, neurite outgrowth, and cell motility.[42] More recently, many synaptic proteins have been shown to be CDK5 substrates, pointing to an involvement of CDK5 in various aspects of synaptic function, such as dopaminergic signaling, neurotransmitter release, and membrane cycling.[40,43,44] Proteins p25 and p29 are equivalent proteolytic segments containing the C-terminal portion of p35 and p39, respectively. Excessive upregulation of CDK5 by the truncated activators contributes to neurodegeneration by altering the phosphorylation state of cytosolic and cytoskeletal proteins, and increased CDK5 activity has been implicated in Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, Niemann-Pick type C disease, and ischemia.[45-48]

The role of CDKs in apoptosis needs to be completely determined. For any antitumor agent to be effective, it will have to not only arrest the proliferation of the cancer cells but also cause their death. Even though a complete picture of how the CDKs function in apoptosis has not been formed, early indications show us that certain CDKs such as CDK1, CDK4, and CDK5 play important roles in apoptosis. It is not yet clear whether inhibition or activation of specific CDKs will have the desired effect on apoptosis. Development of inhibitors that are specific to subtypes of CDK should indicate clearly the ability of the individual CDKs to cause cell death in cancerous cells.

Regulation of Cyclin-dependent Kinases

CDKs encode little more than the protein kinase catalytic core, which is composed of multiple conserved subdomains found in all protein kinases.[49] CDKs are activated in 2 steps. In the first step, the cyclins and other activators p35/p39 bind to the CDKs with the help of their cyclin binding motif (CBM).[50] This enables the CDK to assume an active configuration. Each cyclin binds to a preferred subset of CDKs. The second step consists of phosphorylation of CDKs by CDK-activating complexes such as CAK, which primes the CDKs for their catalytic activity. The CDK is now in a conformation that will allow the formation of peptide-substrate binding. Downstream substrates bind to the activated kinase complex for phosphorylation. The ternary structure of the CDKs provides for specific regions of the protein for binding to the activators, ATP, and substrates.[51,52] Knowledge of the secondary and tertiary structures of these regions and an explicit understanding of the interaction between CDKs and their activators, inhibitors, and substrates will be needed to develop suitable inhibitors that can be specific for each CDK subtype. This review focuses on addressing the specificity and potency of CDK inhibitors from a sequence, structure, and design/discovery (chemistry) point of view.

Sequence Alignment of Cyclin-dependent Kinases 1 to 9

The sequence alignment of the human CDKs (1 to 9) was done using the ClustalW program[53,54] and is shown in Figure 1. The C-terminal residues in CDK1 and the corresponding residues in other CDKs that are not involved in forming the ATP binding pockets are not shown. From the

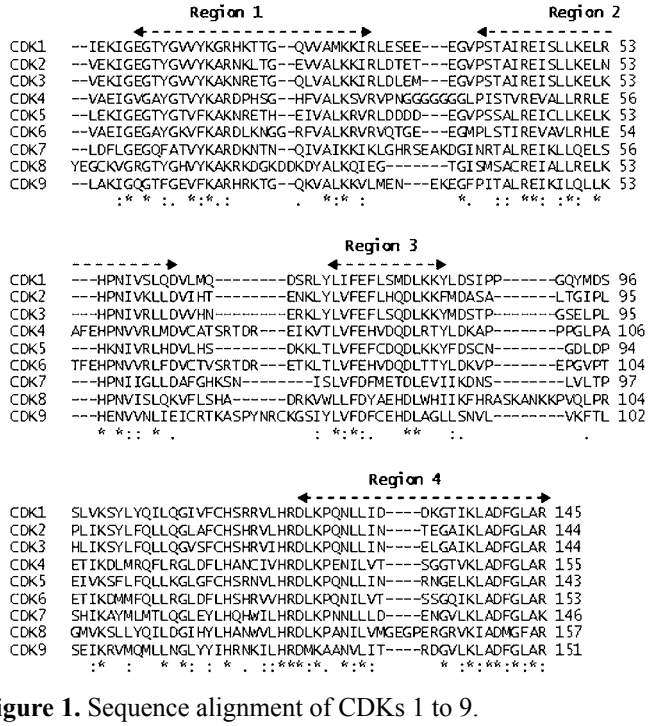

Figure 1. Sequence alignment of CDKs 1 to 9.

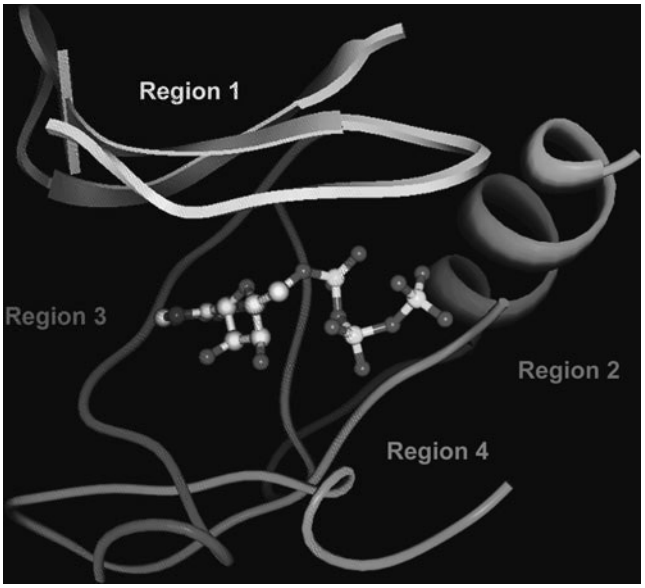

Figure 2. Ribbon representation of the 4 regions that form the ATP binding site of CDK.

reported crystal structure of CDK2-ATP complex,[52] it can be seen that the ATP binding site is formed by 4 contiguous stretches of polypeptide sequences. We labeled the 4 stretches as region 1 (residues 8-35), region 2 (residues 46-67), region 3 (residues 78-90), and region 4 (residues 127-147), as shown in Figure 2. The 4 regions are also marked in the sequence alignment. Region 1 contains the G-rich region; region 2 is the hinge region between the N- and C-terminal loops of the catalytic part of the kinase; region 3 binds to cyclins; and region 4 forms a part of the substrate binding site. The alignment scores among all the kinase protein sequences of CDKs are shown in Table 1. Since the residues in 4 regions form the ATP binding pocket, we calculated the alignment scores for sequences from 1 to 147 (with respect to CDK2) among the CDKs and listed them in parentheses in Table 1. In fact, the residues forming the ATP pocket in the 4 regions have greater homology than the entire sequence alignment scores for all the CDKs. Region 1 of the pocket is a rigid beta sheet structure. The binding of cyclin in order to activate the kinases controls the position of the helix in region 2, and the residues are interacting with the terminal phosphate groups of ATP. Region 3 is the hinge region that is involved in rotating the N-terminal lobe from the C-terminal lobe, thereby creating the substrate binding pocket. ATP and other inhibitors form hydrogen bonds with the residues in the hinge region.

Three-Dimensional Structures of Cyclin-dependent Kinases and Their Complexes

The 3-dimensional (3-D) structures of apo protein (a protein without its coenzymes, cofactors, prosthetic groups that are required for its function) CDKs available in the Protein Databank (PDB; http://www.rcsb.org), with and without phosphorylated residue, and their complexes with ATP with and without divalent ions and cyclins, with natural or synthetic inhibitors, are listed in Table 2. A total of 101 crystal structures of CDKs with resolution and other molecules bound to CDK are listed. Two of these CDK2 structures complexed to substrate peptides, one with ATP inhibitor analog and the other with adenosine diphosphate and nitrate ions, have given valuable insight into the CDK2 substrate binding pocket. In addition, 6 natural protein inhibitors in the unbound form determined by nuclear magnetic resonance (NMR) imaging are listed (shown by italics) in Table 2. The resolution of these crystal structures ranges from 1.3 to 3.1 Å. The majority of the structures reported are for CDK2. Recently, however, a few structures of CDK5 (with ATP, natural and synthetic inhibitors), CDK6 (with natural inhibitors bound to cyclin), and CDK7 (with ATP) have also been reported. Thus, a wealth of knowledge about the ATP binding pocket and the shape and size of the pocket owing to the binding of cyclin for CDK2 is available for structure-based analysis. Neither the crystal nor the NMR structures of CDK1 and CDK4 are available for structural analysis. However, 3 crystal structures of the chimeric structure of CDK2 containing certain critical CDK4 ATP binding residues (7 residues) have been reported.[73] The homology

Table 1. Protein Sequence Alignment Scores (in percentage) for CDKs*

	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9
CDK1	65 (82)	64 (83)	43 (52)	56 (69)	46 (55)	42 (55)	38 (44)	40 (54)
CDK2		75 (87)	44 (55)	59 (75)	48 (60)	44 (56)	37 (45)	39 (56)
CDK3			45 (52)	61 (79)	46 (60)	44 (55)	39 (45)	38 (57)
CDK4				44 (60)	68 (81)	36 (40)	36 (45)	34 (43)
CDK5					46 (62)	45 (55)	36 (44)	41 (60)
CDK6						33 (41)	30 (42)	32 (40)
CDK7							34 (45)	35 (58)
CDK8								29 (45)

*CDK indicates cyclin-dependent kinase. Alignment scores for sequences from 1 to 147 are shown in parentheses.

Table 2. Three-dimensional Structures of CDKs and Their Complexes With Adenosine Triphosphate, Natural and Synthetic Inhibitors*

PDB-ID	R	Sub-type	Cyclin	SMI	PI	NTP	Ions	Oligopeptides	Comments	Ref
1hcl	1.80	CDK2	-	-	-	-	-	-	Apo	55
1pw2	1.95	CDK2	-	-	-	-	-	-	Apo	56
1b38	2.00	CDK2	-	-	-	ATP	Mg++	-	-	57
1b39	2.10	CDK2†	-	-	-	ATP	Mg++	-	-	57
1hck	1.90	CDK2	-	-	-	ATP	Mg++	-	-	58
1f5q	2.50	CDK2	Cyclin	-	-	-	-	-	-	59
1jst	2.60	CDK2†	Cyclin A	-	-	ATP	Mn++	-	-	60
1fin	2.30	CDK2	Cyclin A	-	-	ATP	-	-	-	61
1oiu	2.00	CDK2†	Cyclin A	Yes	-	-	-	-	-	62
1oiy	2.40	CDK2†	Cyclin A	Yes	-	-	-	-	-	62
1ogu	2.60	CDK2†	Cyclin A	Yes	-	-	-	-	-	63
1oi9	2.10	CDK2†	Cyclin A	Yes	-	-	-	-	-	62
1pkd	2.30	CDK2†	Cyclin A	Yes	-	-	-	-	-	64
1fvv	2.80	CDK2	Cyclin A	Yes	-	-	-	-	-	65
1e9h	2.50	CDK2†	Cyclin A3	Yes	-	-	-	-	-	66
1aq1	2.00	CDK2	-	Yes	-		-	-	-	67
1ckp	2.05	CDK2	-	Yes	-	-	-	-	-	68
1di8	2.20	CDK2	-	Yes	-	-	-	-	-	69
1dm2	2.10	CDK2	-	Yes	-	-	-	-	-	70
1e1v	1.95	CDK2	-	Yes	-	-	-	-	-	71
1e1x	1.85	CDK2	-	Yes	-	-	-	-	-	71
1fvt	2.20	CDK2	-	Yes	-	-	-	-	-	65
1g5s	2.61	CDK2	-	Yes	-	-	-	-	-	72
1gih	2.80	CDK2	-	Yes	-	-	-	-	CDK4 inhibitor	73
1gii	2.00	CDK2	-	Yes	-	-	-	-	CDK4 inhibitor	73
1gij	2.20	CDK2	-	Yes	-	-	-	-	CDK4 inhibitor	73
1gz8	1.30	CDK2	-	Yes	-	-	-	-	-	74
1h00	1.60	CDK2	-	Yes	-	-	-	-	-	75
1h01	1.79	CDK2	-	Yes	-	-	-	-	-	75
1h07	1.85	CDK2	-	Yes	-	-	-	-	-	75
1h08	1.80	CDK2	-	Yes	-	-	-	-	-	75
1h0v	1.90	CDK2	-	Yes	-	-	-	-	-	74
1h0w	2.10	CDK2	-	Yes	-	-	-	-	-	76
1h1p	2.10	CDK2†	-	Yes	-	-	-	-	-	76
1h1q	2.50	CDK2†	-	Yes	-	-	-	-	-	76
1h1r	2.00	CDK2†	-	Yes	-	-	-	-	-	76
1h1s	2.00	CDK2†	-	Yes	-	-	-	-	-	76
1jsv	1.96	CDK2	-	Yes	-	-	-	-	-	
1ke5	2.20	CDK2	-	Yes	-	-	-	-		77
1ke6	2.00	CDK2	-	Yes	-	-	-	-	-	77
1ke7	2.00	CDK2	-	Yes	-	-	-	-	-	77
1ke8	2.00	CDK2	-	Yes	-	-	-	-	-	77
1ke9	2.00	CDK2	-	Yes	-	-	-	-	-	77
1oiq	2.31	CDK2	-	Yes	-	-	-	-	-	78
1oir	1.91	CDK2	-	Yes	-	-	-	-	-	78
1oit	1.60	CDK2	-	Yes	-	-	-	-	-	78
1p2a	2.50	CDK2	-	Yes	-	-	-	-	-	79

Continued

Table 2. *Continued*

PDB-ID	R	Sub-type	Cyclin	SMI	PI	NTP	Ions	Oligopeptides	Comments	Ref
1pf8	2.51	CDK2	-	Yes	-	-	-	-	-	80
1pxi	1.95	CDK2	-	Yes	-	-	-	-	-	56
1pxj	2.30	CDK2	-	Yes	-	-	-	-	-	56
1pxk	2.80	CDK2	-	Yes	-	-	-	-	-	56
1pxl	2.50	CDK2	-	Yes	-	-	-	-	-	56
1pxm	2.53	CDK2	-	Yes	-	-	-	-	-	81
1pxn	2.50	CDK2	-	Yes	-	-	-	-	-	81
1pxo	1.96	CDK2	-	Yes	-	-	-	-	-	81
1pxp	2.30	CDK2	-	Yes	-	-	-	-	-	81
1pye	2.00	CDK2	-	Yes	-	-	-	-	-	82
1r78	2.00	CDK2	-	Yes	-	-	-	-	-	83
1urw	1.60	CDK2	-	Yes	-	-	-	-		84
1v1k	2.31	CDK2	-	Yes	-	-	-	-	CDK4 inhibitor	75
1jsu	2.30	CDK2†	Cyclin A	-	P27(Kip1)	-	-	-	-	85
1qmz	2.20	CDK2†	Cyclin A	-	-	ATP	Mg++	Yes	Substrate peptide	86
1gy3	2.70	CDK2†	-	-	-	ADP	Mg++	Yes	Substrate peptide	87
1ung	2.30	CDK5	P25	Yes	-	-	-	-	-	88
1unh	2.35	CDK5	P25	Yes	-	-	-	-	-	88
1unl	2.20	CDK5	P25	Yes	-	-	-	-	-	88
1h4l	2.65	CDK5	-	-	P25(NCK5A)	-	-	-	-	89
1jow	3.10	CDK6	Cyclin	-	-	-	-	-	-	90
1bi7	3.40	CDK6	-	-	P16INK4A	-	-	-	-	91
1bi8	2.80	CDK6	-	-	P19INK4D	-	-	-	-	91
1blx	1.90	CDK6	-	-	P19INK4D	-	-	-	-	92
1xo2	2.90	CDK6	Cyclin V	Yes	-	-	-	-	-	93
1g3n	2.90	CDK6	Cyclin K	-	P18(INK4C)	-	-	-	Cyclin V homolog of Cyclin D	94
1ua2	3.02	CDK7†	-	-	-	ATP	-	-	-	95
1kxu	2.60	-	Cyclin H	-	-	-	-	-	-	96
1bu2	3.00	-	Cyclin	-	-	-	-	-	-	97
1ihb	1.95	-	-	-	P18-INK4C(INK6)	-	-	-	-	98
1mx2	2.25	-	-	-	P18INK4C	-	-	-	F71N	99
1mx4	2.00	-	-	-	P18INK4C	-	-	-	F82Q	99
1mx6	2.00	-	-	-	P18INK4C	-	-	-	F92N	99
1bu9	N/A	-	-	-	P18-INK4C	-	-	-	-	100
1a5e	N/A	-	-	-	P16INK4A	-	-	-	-	101
1ap7	N/A	-	-	-	P19-INK4D	-	-	-	-	102
1d9s	N/A	-	-	-	P15(INK4B)	-	-	-	-	103
2a5e	N/A	-	-	-	P16INK4A	-	-	-	Minimized 1 structure	101
1dc2	N/A	-	-	-	P16INK4A	-	-	-	20 NMR structures	104
1v0b	2.20	-	-	-	-	-	-	-	P Falciparum FPK5 (CDK5)	105
1v0o	1.90	-	-	-	-	-	-	-	P Falciparum FPK5 (CDK5)	105
1v0p	2.00	-	-	-	-	-	-	-	P Falciparum FPK5 (CDK5)	105
1oku	2.90	-	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	106

Continued

Table 2. *Continued*

PDB-ID	R	Sub-type	Cyclin	SMI	PI	NTP	Ions	Oligopeptides	Comments	Ref
1okv	2.30	-	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	106
1okw	2.50	-	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	106
1ol1	2.90	-	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	106
1ol2	2.60	-	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	106
1urc	2.60	-	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	107
1h24	2.50	CDK2†	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	108
1h25	2.50	CDK2†	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	108
1h26	2.24	CDK2†	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	108
1h27	2.20	CDK2†	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	108
1h28	2.80	CDK2†	Cyclin A	-	-	-	-	Yes	Cyclin groove binding	108
1fq1	3.00	CDK2†	-	-	-	ATP	Mg++	-	KAP	109

*PDB-ID indicates Protein Data Bank identification number; SMI, small molecule inhibitor; PI, natural protein inhibitor; NTP, nucleotide triphosphates; Ref, reference number; CDK, cyclin-dependent kinase; Apo, apo protein; ATP, adenosine triphosphate; ADP, adonosine diphosphate; N/A, not available; NMR, nuclear magnetic resonance; FPK, falciparum protein kinase; KAP, kinase-associated phosphatase. Data from the Protein Data Bank (http://www.rcsb.org).
†T160 is phosphorylated.

modeling method was used to generate a homology model of the starfish oocyte *Marthasterias glacialis* CDK1-cyclin B complex from the crystal structure of CDK2-cyclin A using the software program LOOK 3.0.[110-112] This homology model was used to discover a novel set of inhibitors called paullones with nanomolar inhibitory activity against CDK1. Furthermore, a congeneric series of paullones were characterized using a 3-D QSAR with CDK1 inhibition data. Paullones were docked into the ATP binding site of the CDK1-cyclin B models and were optimized with molecular mechanics. Hydropathic analyses of the paullone-CDK1 complexes were performed after the atom types were assigned based on each ligand's electronic properties calculated from quantum mechanics. Hydropathic descriptors formed a significant multiple regression equation that predicted paullone IC50 data. Compounds with low affinity for CDK1 were poor charge acceptors and made less than ideal hydrogen bonding arrangements with the receptor. These considerations led to the prediction that structures such as 9-cyanopaullone would be considerably more potent than the parent compound. The modeling prediction was confirmed by testing these compounds for their inhibitory activity against the purified CDK1 and cyclin B from the starfish oocyte *M glacialis*. Also, 9-nitropaullone emerged as a paullone that had similar potency in enzyme inhibition as well as a favorable anti–proliferative activity profile in living cells. The discovery and design of paullones have clearly established that the use of the homology model of the ATP binding pocket for CDK1 in the structure-based inhibitor design could be easily extended to other subtypes of CDKs.[113]

Adenosine Triphosphate Binding Pocket in Cyclin-dependent Kinases

The ATP binding site of the kinases is a deep cleft formed between the N-terminal and C-terminal lobes of the protein. Apart from bidentate hydrogen bonds formed by the interaction of N-1 and N-6 of the adenine ring of ATP with the backbone carbonyl (81E) and NH (83L) groups in the hinge region of CDK2, owing to the phosphates and sugar moiety, the interactions with ATP are lipophilic. On visually analyzing the ATP binding pocket in the reported[31] crystal structure of CDK2-cyclin A-ATP complex, we identified 16 residues that are important in the binding of ATP. As indicated earlier, the 3 residues at the C-terminal for CDKs are different, and these 3 C-terminal residues are displayed and atom-based color-coded with green for carbon atoms (see Figure 3A). The ATP molecule is shown by the ball-and-stick representation. The molecular surface of these residues (calculated by the molecular surface (MS) program[114]) is shown in Figure 3B. Residues 51E, 80F, 81E, 83L, 84H, 85Q, 86D, 89K, 131N, 137T, and 145D form a sort of "ring" (carbon atoms for these residues are colored cyan) around the ATP molecule, whereas residues 33K and 10I (carbon atoms for these residues are colored gray) interact with the ATP molecule perpendicular to the ring. The molecular surface for the C-terminal residues 296L, 297R, and 298L (PDB code: 1fin) form an additional pocket to the ring. From Figure 3B, it is clear that the ATP molecule interacts with only a few of the residues in the binding pocket and does not interact with the subtype-specific C-terminal residues. In order to have potent and subtype-selective CDK

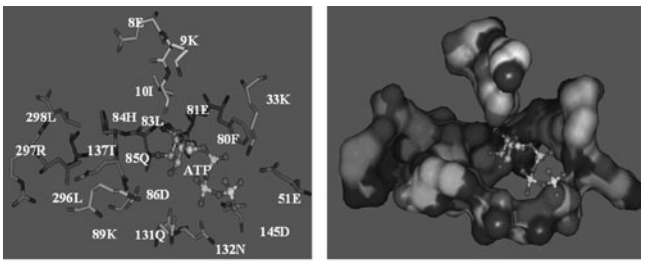

Figure 3. (A) Residues in and around the ATP binding pocket, and (B) Molecular surface of the residues in and around the ATP binding pocket.

inhibitors, the inhibitor should interact with as many residues as possible out of the 19 residues shown in Figure 3A. Modifying adenine base to reach all the residues is difficult because of the 2 fused rings and a lower number of atoms available for chemical modifications.

Adenosine Triphosphate–Competitive Inhibitors of Cyclin-dependent Kinases

The development of small molecule inhibitors of CDKs has progressed rapidly, with all of them targeting the ATP binding site of the kinases and competing with ATP for inhibition. Understanding the basic interactions made by the ATP/inhibitors with the protein has been facilitated by the numerous crystal structure studies. Most of the inhibitors interact on the basis of donor-acceptor motif, similar to ATP. This finding has led to design and development of new classes of inhibitors with modifications targeting specific residues on the protein to improve the potency. Only recently has this effort been directed toward achieving selectivity of the inhibitors to one particular kinase and toward subtype specificity. Several classes of the inhibitors have been successfully modified to improve their potency in the inhibition of CDKs, and some of them have even showed moderate to significant subtype selectivity.

The first generation of inhibitors relied on similarity of interactions of known inhibitors with analogous structures. The purine ring system is used in several CDK inhibitors. The initial purine analogs exemplified by olomoucine[115] showed good potency for CDK1, CDK2, and CDK5 but were not active for CDK4 and CDK6. Further development of new libraries of analogs with bulky substituents at the 2 and 9 positions showed a tremendous increase in potency, while maintaining a similar selectivity profile illustrated by roscovitine.[116] Inhibition of G_1/S phase and G_2/M/G_1 phase progression was observed for both olomoucine and roscovitine in a dose-dependent manner.[117,118]

Staurosporine, a metabolite from *Streptomyces sp,* is a natural ATP-competitive inhibitor of kinases.[119,120] It was initially identified as a potent inhibitor (IC50 = 1 nM) of protein kinase C. It was later established that staurosporine is a nonspecific inhibitor of kinases that blocks CDK1-cyclin B with an IC50 of 3.2 nM. Some of the derivatives of staurosporine, such as UCN-01, K252C, and CGP41251, show excellent antitumor activity against different cancer cell lines.[121,122] UCN-01 has been shown to reduce the amount of phosphorylated pRb, thereby blocking the G_1/S transition, and is in clinical trials. The nonspecificity of these compounds has presented the possibility of non-mechanism-dependent side effects in clinical use. One other class of compounds that has been extensively studied is the flavones. Flavopiridol and deschloroflavopiridol are naturally occurring alkaloids showing cytotoxic properties against tumor cell lines.[123,124] Flavopiridol is the first CDK inhibitor to enter clinical trials. Flavopiridol has highest activity for CDKs and GSK3β and is moderately more selective for CDK4, CDK6, and CDK1 than for CDK2. It has also been shown to inhibit CDK7-cyclin H complex and P-TEFb complex containing CDK9-cyclin T1. Flavopiridol has shown promising results in combination therapy, enhancing the activity of paclitaxel in human trials.

The pyrimidines[125] and pyrido[2,3-days]pyrimidinones[126] series of inhibitors have been designed on the basis of purine-based derivatives. CINK4,[127] a pyrimidine analog, was identified in a high-throughput screening as a CDK4 (IC50 = 1.5 μM) and CDK6 (IC50 = 5.6 μM) inhibitor. It also inhibits CDK5 (IC50 = 25 μM) to a lesser extent, while inhibition of CDK2 and CDK1 is accomplished at only very high concentrations (above 50 μM). Phenylpyrimidine CGP60474 is a potent inhibitor of CDK1 and CDK2, with weaker inhibition of CDK4. Pyrido[2,3-days]pyrimidinones showed potency for receptor tyrosine kinases as well as CDKs. Many modifications of the core structure have led to compounds that have different selectivity profiles but are more potent.

Oxindoles[128] (eg, indirubin) have been used in traditional Chinese medicine for the treatment of chronic diseases such as leukemia. Several derivatives of oxindoles have been synthesized to improve their bioavailability and potency. Analogues such as indirubin sulfate and indirubin monoxime have shown good activity and moderate selectivity for CDK1, CDK2, and CDK5 as compared with CDK4. Three-substituted oxindole was able to inhibit CDK4 with moderately more selectivity over CDK1 and CDK2; and 3,5-disubstituted oxindole derivative SU9516 showed higher selectivity for CDK2 and CDK1 over CDK4. Some hydrazone analogs[129] have been reported to have high potency of inhibition for CDK2 and CDK1. Paullones are another class of compounds that have shown good potency for CDK inhibition, and most of them show good selectivity for CDK1, CDK2, and CDK5 and almost negligent activity for CDK4.[130] The nitro derivative alsterpaullone and the cyano derivative improved the potency of this class of compounds.[131]

Diarylurea derivatives were designed by Honma[132] on the basis of structural information derived through homology modeling of CDK4. A diverse set of compounds were synthesized, some of which showed very high potency and good selectivity for CDK4. The IC50 against CDK subtypes for 30 diverse cores are listed in Table 3. The 2-D chemical structures of these 30 compounds are shown in Figures 4A and 4B. The details of the activities of the analogs of these core structure inhibitors for CDKs have been reviewed.[148,149]

Cyclin-dependent Kinase Inhibitors Represented by Reported Protein Data Bank Structures

Only a few classes of inhibitors have been reported as complexed in a crystal structure. Purines depicting various substituents at different positions—such as purvalanol B, roscovitine, the NU series of inhibitors (eg, NU6058, NU6094, NU6102, NU2058, NU2067), and H717—constitute the major class of inhibitors that have been successfully cocrystallized with CDK2. The second class of inhibitors found in the crystal structure database to a considerable extent is the pyrimidines. Table 4 lists the class of known CDK inhibitors reported in crystal structures and their PDB identification numbers.

For structure-based drug discovery and design, it is important to quantify the ligand-protein contacts. We used the occluded surface method, which was developed in our laboratory and has been used successfully to identify critical ligand-protein interaction,[150,151] to scoop the residues that are in contact with ligands in a 3-D structure of CDK-ligand complex. We have identified critical residues that are involved in the binding of natural inhibitors such as p27 and

Table 3. Selected Small Molecule Inhibitors With IC50 (μM) Values Against CDK Subtypes (1-6)*

	CDK1	CDK2	CDK4	CDK5	CDK6	Reference
1	7.000	7.000	>100	3.000		133
2	0.450	0.700	>100	0.160		116
3	>50	>50	1.500	25.000	5.600	127
4	0.030	0.170	0.100			123
5	0.020	0.050	~10			122
6	0.055	0.035	0.300	0.065		128
7	0.040	0.022	0.200			
8	0.035	0.015	>10	0.040		131
9	0.120	0.078	0.042			132
10	1.800	0.440	0.002			132
11	0.600	1.500	1000.000			134
12	0.130	2.110	6.150			134
13	2.800	4.500				135
14	12.000	0.540				135
15		0.600	>2.1			136
16		1.200	8.300			136
17	1.600	1.300				137
18	0.005	0.001				78
19		0.032	0.150			78
20		0.006	2.700			138
21	0.006	0.009	0.230			139
22	2.900	2.200				140
23	0.104	0.033	0.810			141
24	0.055			0.005		142
25		0.009		0.005		143
26	0.015	0.005	0.260			84
27	0.279	0.037	>10	0.114		144
28	0.160	0.250	1.290			145
29	5.000			25.000		146
30	0.001	0.002	0.029			147

*CDK indicates cyclin-dependent kinase.

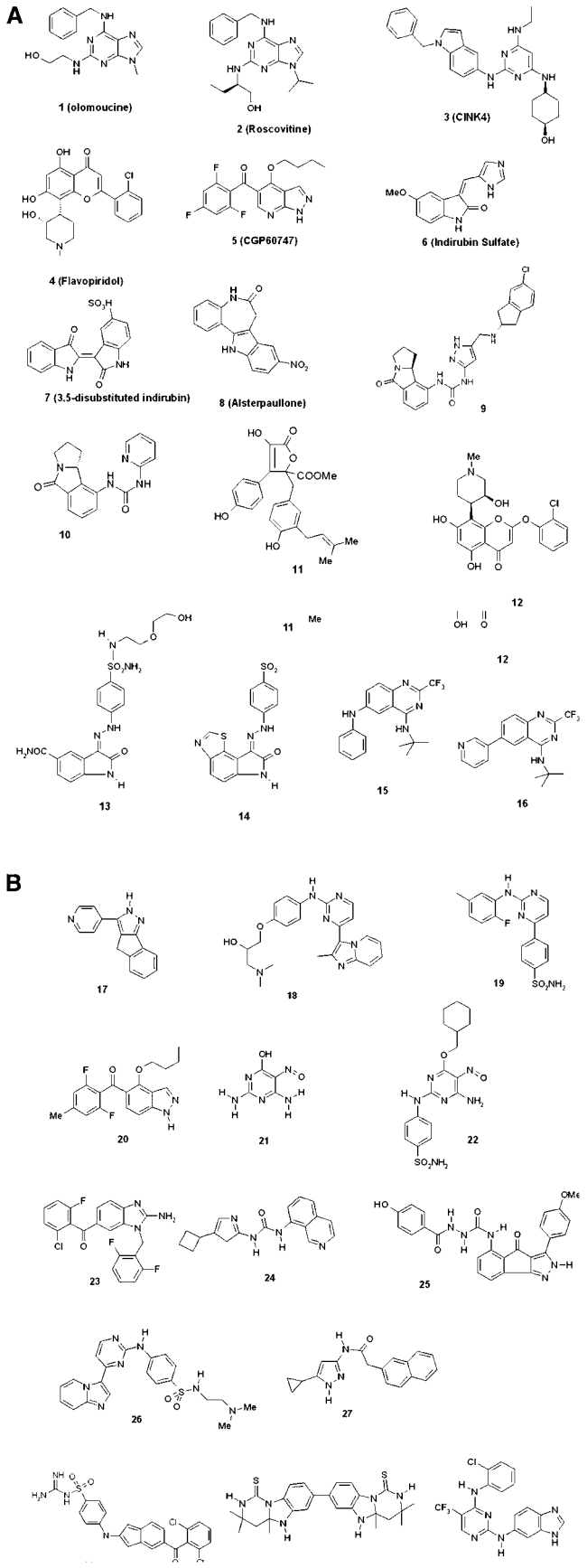

Figure 4. (A) Selected CDK inhibitors, and (B) Selected CDK inhibitors.

ATP. As examples, we have summarized the results obtained from the occluded surface method for 9 crystal structures of CDK2 complexes (see Table 5). The chemical structures of these inhibitors are shown in Figure 5. The higher the values were, the better the contact between the CDK and the ligand. In this table, the natural protein inhibitor makes contacts with 48 residues in CDK involving the ATP binding site. Because these inhibitors were designed for the ATP binding pocket, they make contacts with residues only in the ATP binding pocket. Residues I10, G11, G13, V18, K33, F80, F82, L83, Q85, D86, K89, Q131, L134, D145, and L148 make significant interactions with either small molecule inhibitors or ATP. It should be noted that compound (1URW) has groups interacting outside of the ATP pocket. It is of interest to note that only the natural protein inhibitor and the compound (1fvv) have contact with the critical E51 residue. None of the small molecule CDK inhibitors resemble the oligopeptides (PEFYYR) of the inhibitor p27 that occupies the ATP pocket.

Designing Specific Inhibitors for Disrupting the Interaction Between Cyclin Ds and Retinoblastoma Protein

Two classes of cyclins get successively activated during the G_1 phase of the cell cycle, D-type cyclins (cyclins D1, D2, and D3)[152] and cyclin E (cyclins E1 and E2).[153] Expression of D-type cyclins represents a fundamental link between mitogen, nutrient stimulation, and the cell cycle machinery and is the underlying cause of many diseases, such as cancer.[154] Cyclin D binds CDK4 or CDK6 to activate the kinase activity of these proteins.[155] The extent to which the cyclin subunit contributes to CDK activity beyond the role as an essential activator is being studied intensely by various groups.

Recent x-ray crystallographic studies[108] on CDK2-cyclin A complexes have elucidated the structural basis of one mechanism by which the cyclin subunit enhances CDK substrate phosphorylation. Through a comparison of sequences of proteins (ie, E2F1, p107, p130, pRb, and members of Cip/Kip CDK inhibitor family), a CBM, ZRXL, in which Z and X are typically basic, has been identified.

In a recent study,[106] a series of peptides (ZRXLYY', where Y and Y' are hydrophobic residues) containing nonnatural amino acids were prepared using the CBM present in the tumor suppressor proteins p21 and p27 as a template. These were shown to have nanomolar to micromolar CDK2 inhibitory activity by binding to cyclin A (Table 6). The cyclin A binding site comprised a groove resulting from the exposed surface of the cyclin A α1, α3, and α4 helices. Several subsites lining the hydrophobic pocket (Met210, Ile213, Leu214, Trp217, Leu253, Glu220, Val221, Ile281) contribute toward the hydrogen bonding of the ligand.

Table 4. CDK Inhibitor Group and Their PDB IDs*

Class of Inhibitors	PDB IDs
Purines	1CKP, 1E1V, 1G5S, 1G28, 1HOW, 1HOU, 1HOV, 1H1P, 1H1Q, 1H1R, 1H1S, 1OI9, 1OIU, 1OIY, 1UNL
Pyrimidines	1DI8, 1E1X, 1H00, 1H08, 1H07, 1OGU, 1OIQ, 1OIR, 1OIT, 1PXI, 1PXJ, 1PXK, 1PXL, 1PXM, 1PXN, 1PXO, 1PXP, 1URW
Oxindoles	1E9H, 1FVV, 1FVT, 1KE5, 1KE6, 1KE7, 1KE8, 1KE9, 1PF8, 1PYE, 1R78, 1UNG, 1UNH
Staurosporine analogs	1AQ1, 1PKD
Urea analogs	1GIH, 1GII

*CDK indicates cyclin-dependent kinase; PDB-ID, Protein Data Bank identification number.

Table 5. Residues in CDK2 That Are in Contact With Ligand/Inhibitor Molecules*

	PDB†	ID†	1jsu	1aq1	1ckp	1fvv	1g5s	1hck‡	1pxo	1urw	1w0x
	3	N								4	
►	4	F								1	
	8	E				1					
	9	K				3					
	10	I		16	14	25	15	12	9	19	24
	11	G		5			2	3	1		1
	12	E		2			3	2			
	13	G	1	5			4	8			
	14	T	13				1	7			
	15	Y	3					2			
	16	G	11								
	17	V	25								
	18	V	30	14	4	9	15	16	4	9	4
	19	Y	56							2	
	20	K	25				1				
	21	A	22								
	22	R	12								
	23	N	21								
►	24	K	1								
	28	E	1								
	30	V	10								
	31	A	5	7	7	6	7	6	6	7	7
	32	L	17								
	33	K	20	5		6	2	6	11	2	
	34	K	2								
►	35	I	2								
►	45	P	3							3	
►	47	T	7							3	
	50	R								2	
►	51	E	1			1					
►	64	V	2	3	3	5	6	1	6	3	3
►	67	L	6								
	68	D	7								
	69	V	1								
	70	I	30								

Continued

Table 5. *Continued*

	PDB†	ID†	1jsu	1aq1	1ckp	1fvv	1g5s	1hck‡	1pxo	1urw	1w0x
	71	H	6								
►	72	T	5								
►	75	K	7								
►	77	Y	27								
	79	V	12								
	80	F	10	10	7	12	15	1	10	9	4
	81	E	3	3	3	1	2	2	2	2	2
	82	F	13	8	8	7	12	5	6	9	13
	83	L	7	7	7	6	8	6	8	9	8
	84	H	4	3	5	4	4		2	4	4
	85	Q	5	3	7	11	3		3	9	3
►	86	D	4	8	9	10		4	9	11	8
►	89	K			4	7			5	8	3
	91	M									
►	92	D								4	
	127	D	2					1			
	128	L									
	129	K	1					7			
	130	P									
	131	Q	4	9			2	15	3	1	6
	132	N	3	3			4	3	2	1	
	133	L									
►	134	L	14	22	14	13	13	11	18	15	17
	143	L									
	144	A	4	6	1	4	4	1	7	6	1
►	145	D	11	8		7	12	4	7	8	
	147	G									
	148	L	10	1						1	

*CDK indicates cyclin-dependent kinase; PDB, Protein Data Bank; ID, identification number. ► denotes a break in the sequence number. The values in the table are weighted occluded surface area between ligand and each residue in CDK2.
†Bold highlighted residues make significant contribution to the ligand-protein interactions.
‡T160 is phosphorylated.

Table 6. Structure-Activity Relationships*

Sequence **R – X – L – Y – Y'**	IC_{50} (μM)[108] Competitive Cyclin A Binding	 CDK2/Cyclin A Kinase Activity
R – R – **L** – N – (*p*-F-Phe) – NH_2	0.53	7.2
Cit – Cit – **L** – A – (*p*-F-Phe) – NH_2	21	37
R – Cit – **L** – I – (*p*-F-Phe) – NH_2	0.59	0.52
H – A – K – **R** – R – **L** – I – F – NH_2	0.05	0.14
Cit – Cit – **L** – A – (*p*-F-Phe) – NH_2	79	12
R – R – **L** – I – F – NH_2	0.68	7.7
Ac – **R** – R – **L** – N – F – NH_2	12	>50
Ac – **R** – R – **L** – N – (*m*-Cl^-Phe) – NH_2	5.6	31
Ac – **R** – R – **L** – N – (*p*-Cl^-Phe) – NH_2	1.8	13

*CDK indicates cyclin-dependent kinase; Cit, isosteric substitution of Arg (ie, replacement of guanidine). Bold residues are critical for binding.

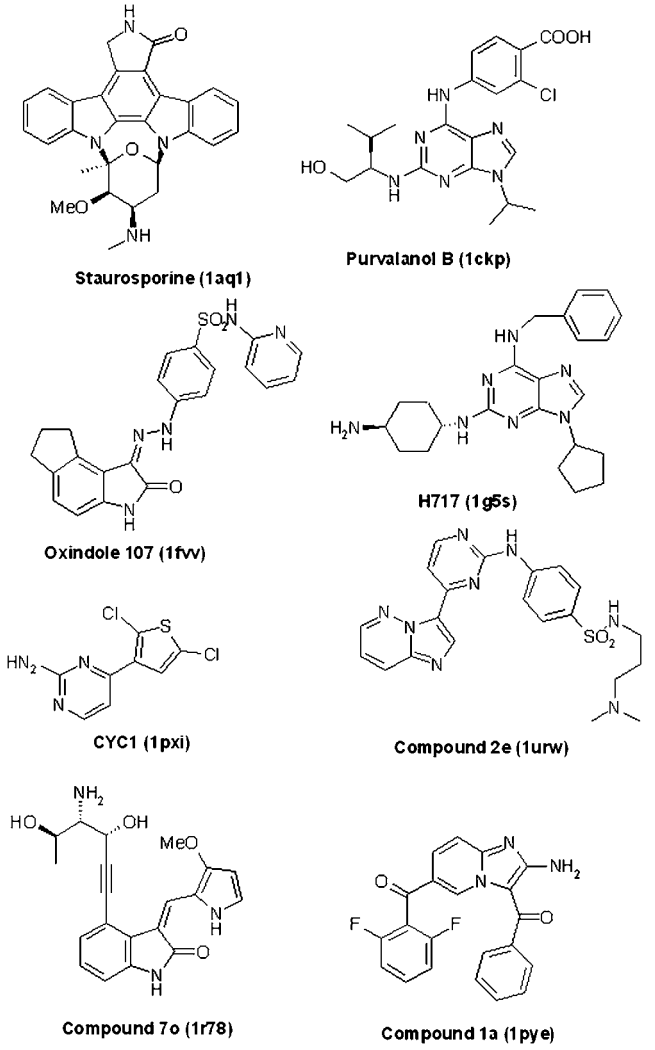

Figure 5. Selected structures from PDB.

Figure 6. Sequence alignment of CDKs 1, 2, 4, and 5. "*" represents fully conserved residue; ":" represents strong conservation; and "." represents weak conservation.

Figure 6. The residues (167-276) in CDK1 and the corresponding residues in other CDKs that are not involved in forming the ATP binding pockets are not shown. As stated earlier, the ATP binding pocket can be divided into 4 regions, namely, the G-rich region 1, the cyclin binding region 2, the hinge region 3, and the substrate binding region 4. The alignment scores among the entire kinase protein sequences of CDKs are shown in Table 1. It is clear that CDK1 and CDK2 have the closest homology. In fact, the residues forming

Benzeno et al[156] have found that Kruppel-like factor (KLF6), a tumor suppressor gene, mediates growth inhibition through an interaction with cyclin D1, leading to reduced phosphorylation of pRb at Ser795. Furthermore, the growth-suppressive activity of KLF6 is linked to p53-independent transactivation of p21, a key CDK inhibitor. KLF6 disrupts cyclin D1-CDK4 complexes and forces redistribution of p21 onto CDK2, which promotes G_1 cell cycle arrest.[157] The KLF6 sequence predicts consensus sites for both a CDK phosphorylation site at Ser171 and a proximal cyclin consensus binding site at the COOH terminus (ZRXL motif, Z = basic residue, at amino acids 279-283). Benzeno et al[156] have described an interaction between the tumor suppressor protein KLF6 and cyclin D1, defining a new mechanism of KLF6-mediated growth suppression.

Potency and Cyclin-dependent Kinase Subtype Selectivity

The sequence alignment of the human CDKs (1, 2, 4, and 5) was done using the ClustalW program[52-54] and is shown in

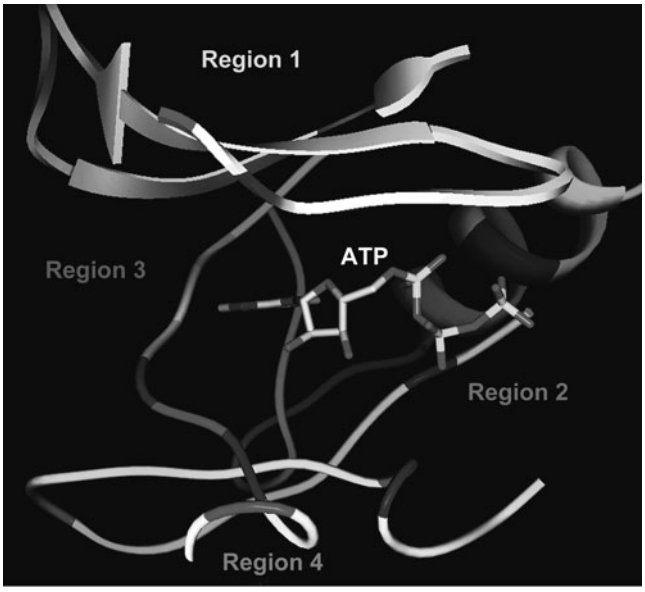

Figure 7. Ribbon representation of the potential residues that are critical for increasing the potency and specificity.

Table 7. Residues To Be Targeted for CDK Subtype Selectivity*

CDK1	CDK2	CDK4	CDK5
E8	E8(1)	A10	E8
M85	Q85(3)	Q98	Q85
K89	K89(3)	T102	K89
Q132	Q131(4)	E144	Q130
D138	T137(4)	S150	R136
291-DNQIKKM--297	291-TKPVPHLRL-298	299-EGNPE---303	289-FCPP-----292

*CDK indicates cyclin-dependent kinase. The sequence in the last row is the C-terminus.

the ATP pocket in the 4 regions have greater homology than the entire sequence alignment scores between CDKs 1 and 2. By comparing the residues constituting the ATP binding pocket, we can identify those residues that can improve the potency and the subtype specificity. In the reported crystal structures of CDK2, ATP and the majority of inhibitors form 2 to 3 hydrogen bonds with the residues in the hinge region. Using the alignment of CDK sequences as shown in Figure 6 and the crystal structure of CDK2-cyclin-A-ATP complex, we have identified 3 groups of residues in the ATP binding pocket for binding the inhibitors. The first group of residues (shown by a green ribbon in Figure 7) is involved in the formation of 2 to 3 hydrogen bonds with the inhibitors. The second group of residues (shown by a blue ribbon) is important for increasing the potency of the inhibitors. And the third group of residues (shown by a magenta ribbon) is for increasing the CDK subtype selectivity of inhibitors. The potential residues that will increase the potency of the CDK inhibitors are shown by a blue ribbon, and the residues that will increase the selectivity of CDK inhibitors are shown by a magenta ribbon. The residues that are identified as increasing the potency of CDK inhibitors are listed in Table 7. Also, the side-chain or backbone atoms with which the inhibitor might make favorable interactions and the region to which these residues belong are listed. For residues K9, E81, L83, H84, and E131, only backbone atoms are identified. For residues E8, A85, D86, K29, and N132, only the side-chain interactions are identified. For residues I10 and D145, both backbone and side-chain atoms are identified as potential interacting atoms. In Table 8, the residues that are identified as the potential sites for increasing the subtype selectivity are listed. The number in the parentheses denotes 1 of the 4 regions to which the particular residue belongs. From the sequence alignment shown in Figure 6, the specificity could be achieved by interacting with the C-terminal residues because of the difference in length as well as sequences between CDKs. As shown in Table 7, CDK2 has the longest C-terminal among the 4 CDKs. In fact, in the crystal structures of CDK2, the residue at the C-terminal is in close proximity to the hydrogen bonding residues to ATP. Because of this, it is possible to make subtype-specific compounds by adding appropriate groups to interact with the C-terminal residues.

Conclusions

In this review, we have addressed the issue of selectivity and potency of CDK inhibitors based on the sequences of CDK subtypes and their interactions with small as well as natural protein inhibitors. To increase the subtype selectivity for CDK, the inhibitors should be designed so that the chemical groups are able to reach residues outside of the ATP binding sites. Also, discovery/design of novel, small, and flexible cores mimicking not ATP but the pharmacophore of natural protein inhibitor p27 will improve the potency and selectivity of CDK inhibitors. Future design work should include residues involved in the binding site of the ATP triphosphate (in particular the γ-phosphate group). With these inhibitors, it might be possible to reach residues in CDK that are specific for substrates.

Table 8. Residues To Be Targeted for Increasing the Potency of Cyclin-Dependent Kinase Inhibitors

Residue	Side-chain	Backbone	Region
E8	Yes	-	1
K9	-	>C = O	1
I10	Yes	>C = O	1
K33	Yes	-	1
E51	Yes	-	2
F80	Yes	-	3
E81	-	>C = O	3
L83	-	−NH & >C = O	3
H84	-	>C = O	3
Q85	Yes	-	3
D86	Yes	-	3
K89	Yes	-	3
E131	-	>C = O	4
N132	Yes	-	4
D145	Yes	−NH	4

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